Reproduction and early stages of development in two species of Australian rainbowfishes, *Melanotaenia nigrans* (Richardson) and *Melanotaenia splendida inornata (Castelnau).*

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ABSTRACT

Reproductive behaviour, colouration of adult fish and development to hatching of *Melanotaenia nigrans* and *Melanotaenia splendida inornata* were studied under laboratory conditions during summer and autumn (February to early May). At 26°C ± 1°C, all observed pairs of fish spawned each day during this period. The eggs of the two species differed in size as well as in the number and colour of oil droplets. The rate of embryonic development under identical conditions of temperature and light was found to be almost parallel in the two species. However, the melanophore pattern of the developing embryos was found to be sufficiently different to identify each species. At hatching, the larvae were well developed, with functional mouth and well formed pectoral fins.

INTRODUCTION

Melanotaenia nigrans (Richardson, 1843), the black striped rainbowfish and *Melanotaenia splendida inornata* (Castelnau, 1875), the checkered rainbowfish, belong to the family Melanotaeniidae, a group of freshwater fishes endemic to Australia, New Guinea and the Aru Islands.

Although most species of melanotaeniids have been kept and bred in captivity since the early part of this century, little is known of their life history or their biology. Sterba (1963), Breder and Rosen (1966), Axelrod *et al.* (1971), Lake (1978) and Munro (1980) give brief notes on reproduction of some melanotaeniids whilst Beumer (1979), has published a short account of spawning behaviour, incubation period and size of prolarvae at hatching for *Nematocentris splendida* (= *Melanotaenia splendida splendida,* see Allen, 1980). K. A. Bishop (pers. comm.), who has studied the ecology of fish of the Alligator Rivers region, suggests that melanotaeniids in that region may either spawn daily for a period of about four to five months, or may spawn opportunistically if conditions are favourable. Beumer's findings (1979) confirm this view for the species of rainbows he studied in northern Queensland. Factors initiating reproductive behaviour in rainbowfishes are not known but Beumer (1979) does suggest day-length could be a factor which may induce spawning.

Colour variability in melanotaeniids has been a source of confusion to students studying their life history and to taxonomists alike. Colour appears to vary from population to population of the same species as well as within a population, particularly during different stages of the fishes' life. Most of the rainbowfishes are also sexually dichromatic and this adds further difficulty to species recognition. Lake (1978) has recorded some of this variability in relation to age, sex, stress, habitat and during spawning. Munro (1980) has also given a description of several colour phases for two species of melanotaeniids from the south-eastern coast of Australia. The most recent of many references to spawning in rainbowfishes (Lake, 1971, 1978; Munro, 1958, 1967, 1980; and Beumer, 1979) record some aspects of sexual display but embryonic development and larval and juvenile growth are not discussed.

MATERIALS AND METHODS

Adult fish in breeding condition were collected in Northern Territory in late spring, between 17-20 November, 1980. Specimens of *M. nigrans* were taken from a small permanent water-hole at Koongarra (12°36′S 132°52′E) approximately 40 km south-east of Mudginbarry. *M. s. inornata* was collected in Ja Ja Lagoon, (12°32′S 132°53′E) about 10 km north of Mudginbarry Homestead. The fish were air freighted to Sydney, 3 to 4 fish to one small plastic bag containing about 400 mls of well oxygenated water. On arrival at Macquarie University, the fish were transferred to 200 litre tanks in which the water had been allowed to stand for one week.

Small amounts of frozen brine shrimp were fed to the fish on the day of arrival and dried commercial food (TetraMin brand) was given on the following day. A regime of three feeds a day was established (dry food twice a day and frozen brine shrimp once a day) as soon as the fish began accepting food. After three months, breeding pairs were selected and moved into separate 30 litre tanks containing fine leafed weed e.g. *Cabomba* sp. The temperature of the tanks was maintained at 26° ± 1°C.

The breeding tanks were cleaned every two to three weeks. Untreated tap water was found to be quite satisfactory whenever addition or change of water was necessary. Fungal infection, when it occurred, was treated by placing the fish in a separate 30 litre tank in which 7 drops of 1% Methylene Blue had been added to the water. The fish were subjected to this treatment for up to two weeks.

Fish spawned amongst weed and the eggs were removed as soon as possible to another 30 litre tank to prevent predation by adults. On hatching, the fry were fed Liquifry No. 1 and TetraMin Baby Fish Food "E" five times a day. From the end of the second week brine shrimp nauplii were also given to the developing young to supplement their diet.

Eggs removed for study were transferred to 90 mm diameter covered plastic petri dishes in which a drop of 0.5% Malachite Green solution had been added to the water to minimize fungal infection. The petri dishes were moved to a thermally insulated room where the temperature was kept constant at 25° ± 1°C and the photo-period maintained at 12 hours daylight per 24 hours throughout the period of incubation.

The behaviour, colour changes and spawning were observed and recorded for each pair of adult fish of both species. For convenience, the observed embryonic development was recorded in 24 stages, selected to point out and highlight some of the changes taking place. All measurements were made using an Olympus dissecting microscope with a graticule eye-piece. Heart rates of developing embryos were counted from first observed pulsations of the pericardial cavity up to the time of hatching.

RESULTS

COLOURATION AND BEHAVIOUR OF ADULT FISH DURING THE PRE-SPAWNING AND SPAWNING PERIOD

In aquarium conditions, pre-spawning behaviour usually occurs in the morning and may continue for up to an hour before spawning takes place. Initially the fish remain stationary, facing in the same direction, with their bodies apart but with the caudal fins touching, the caudal and pectoral fins fanning gently. During this period (5 to 10 minutes) the colours in both sexes become more intense but to a lesser extent in the female.

In *M. nigrans* males, the nape and pectoral fins become pale luminous orange, the dorsal fins bright yellow edged with black and the caudal, a deep sulphurous yellow. The anal and pelvic fins remain pale but are edged with red and black. The red colour extends along the body ventrally from the branchiostegals to one third of the way along the ventral edge of the caudal fin. The dark midlateral stripe which extends from the snout to the base of the caudal fin becomes prominent and a few red flecks appear on scales just below the stripe near the tail. Above the midlateral stripe, the body darkens from yellow-grey to very dark grey except for the area posterior to the dorsal fins where it becomes golden. Below the midlateral stripe, the body is iridescent mauve. In the female the colours are paler. The caudal fin is yellow and the midlateral stripe is prominent as in the male. The edges of the dorsal and anal fins are not coloured. In both sexes, a faint red spot is present on the operculum.

In *M. s. inornata* males, the edges of the fins and the checkering of the caudal and second dorsal fins become black. The nape may darken to black in some but not all fish. The midlateral stripe is prominent, extending from the origin of the pectoral fin to the caudal peduncle. Near the tail, two shorter dark lines appear above and below the midlateral stripe. The normal orange-yellow longitudinal stripes become a more intense colour in both sexes. The males have an iridescent purple sheen in light. The operculum has a glowing red spot in both sexes. The pectorals do not become coloured in either males or females. In the females, the midlateral stripe darkens before spawning but the stripe is not as long as in males. The checkered appearance of the second dorsal fin becomes more noticeable and the colour of all the fins darkens except in the case of the pectorals.

Chasing follows immediately after the colour change. The males chase the females, swim below them and brush their vent area with erect dorsal fins or butt them in the vent region or in the area of the pectoral fins. The males frequently shake themselves as they swim below the females. They display erected fins as they swim beside or at right angles in front of the females. During the pre-spawning chasing and display, the males can become quite aggressive and nip the females if the latter do not show interest in the display. In the final phase of pre-spawning behaviour, the pair swim with their bodies parallel, sinking and rising and then remain in one place with heads touching and their bodies vibrating rapidly. After 3 to 5 seconds of this, eggs and milt are shed, the male swishes its tail and the eggs are seen swirling in the wake. Spawning usually occurs in the top 100 mm of the tank.

In aquarium conditions, spawning occurred daily during the summer months, almost always in the morning. However, if males and females in breeding condition, previously kept separately, are placed together, they may spawn late in the day. In such cases, preliminary pre-spawning behaviour may not occur.

TABLE 1. Comparison of biological data obtained from examination of eggs of *M. nigrans* and *M. s. inornata.*

	M. nigrans	*M. s. inornata*
Number of eggs examined	6	6
Egg diameter (mm)	1.05 (1.00-1.08)	0.88 (0.87-0.92)
Perivitelline space	0.05 (0.04-0.06)	0.05 (0.04-0.06)
No. oil droplets	45-55	55-65
Oil droplet diameters (mm)	0.02-0.07	0.01-0.08
Oil colour	Chartreuse	Golden
No. eggs per spawning	*50-70	*60-100

*Accurate counting of the eggs is difficult as the male disperses the eggs rapidly by the swishing of his tail. Also, many of the eggs are eaten before and after they attach to the weeds or other objects.

THE EGGS

The eggs of both species are similar, the major differences being in their size and in the number and colour of the oil droplets, (Table 1).

In both species studied, at spawning, the eggs are spherical and opaque with many filaments originating from one small area of the chorion. The filaments are straight and 20-30 mm in length. On coming into contact with a surface which is not smooth, such as weed, the filaments adhere and contract so that the eggs become suspended. The eggs, which become clear a few seconds after spawning, are large and have numerous small oil droplets at the pole where the filaments occur. The oil droplets appear to aid flotation of the eggs until they attach to an object by their filaments. The oil droplets move down the periphery of the egg to the opposite pole while the first four cleavages of the developing embryo take place.

The chorion is clear, smooth and colourless, and the yolk is also colourless and non-granular. The oil droplets do not coalesce, although they remain in the same position during the development of the embryo and diminish in number. Some are still apparent in the yolk sac just prior to hatching.

EMBRYOLOGY

The eggs of *M. nigrans* and *M. s. inornata* are telolecithal. Division is meroblastic and restricted to a small disc directly below the filaments. The yolk material remains undivided and is later covered by the periblast and enclosed by ectoderm.

Orderly movement of oil droplets towards the mid-polar periphery indicates that the eggs are fertilized. In unfertilized eggs, the oil droplets become randomly scattered within thirty minutes, and the yolk becomes a dense cloudy mass.

The general pattern of teleost embryo development is followed as discussed by Breder (1959), Armstrong and Child (1965), Llewellyn (1973, 1974) and Long and Ballard (1976). In the present study, the hatching prolarvae are more advanced embryonically than those studied by the above workers. At hatching the yolk sac is very reduced, the pectoral fins and mouth are present and functional and the prolarvae swim strongly. Feeding commences within 24 hours.

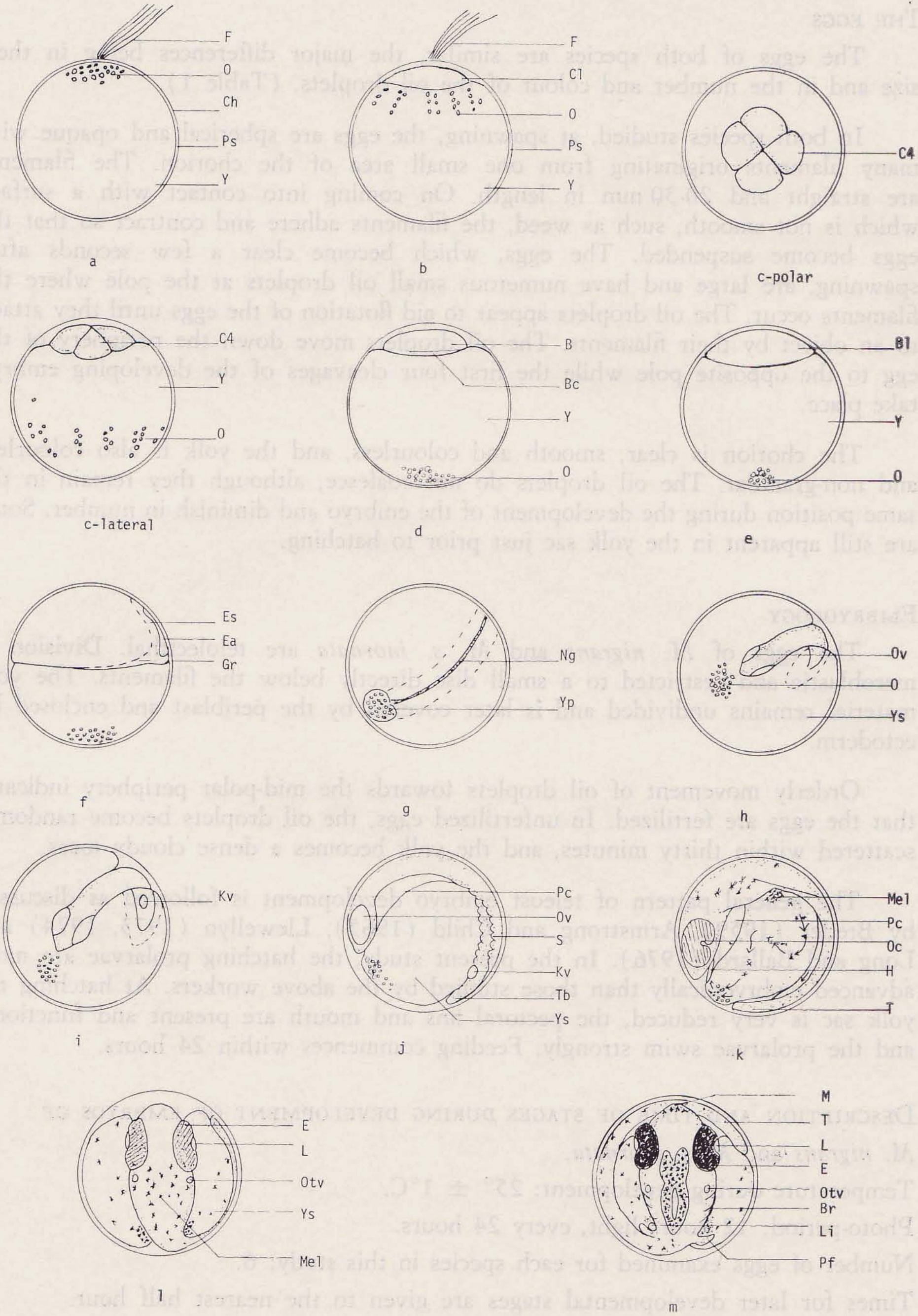
DESCRIPTION AND TIME OF STAGES DURING DEVELOPMENT OF EMBRYOS OF *M. nigrans* and *M. s. inornata*.

Temperature during development: 25° ± 1°C.

Photo-period: 12 hours light, every 24 hours.

Number of eggs examined for each species in this study: 6.

Times for later developmental stages are given to the nearest half hour.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Stage	Time	
	M. nigrans	*M. s. inornata*
1. Fertilized eggs. Oil droplets are directly below the filaments. (Fig. 1a).	0-5 s.	0-5 s.
2. First cell forms. Oil droplets show orderly movement towards the mid-polar periphery. (Fig. 1b).	0.66 h.	0.55 h.
3. Two cell stage. Oil droplets at mid-polar periphery of yolk.	1.5 h.	1.25 h.
4. Four cell stage. Oil droplets continue downward movement towards vegetal pole. (Fig. 1c).	2 h.	2 h.
5. Eight cell stage. Most oil droplets have reached the vegetal pole.	2.5 h.	2.5 h.
6. Sixteen to thirty two cells. Oil droplets are all at vegetal pole where they remain. They do not coalesce. (Fig. 1d).	3-4 h.	3-4 h.
7. Start of epiboly. Blastoderm flattens out and begins spreading downwards over yolk. (Fig. 1e).	5 h.	5 h.
8. Embryonic shield and germ ring visible. Blastoderm continues downward movement. (Fig. 1f).	14 h.	14 h.
9. Neural plate and groove visible. The yolk is now enclosed by the blastoderm except for the small yolk plug. (Fig. 1g).	20.5 h.	21.5 h.

Fig. 1. Embryonic development of *M. nigrans* and *M. s. inornata.* (a) Fertilized egg, 0-5 s; (b) First cell stage 0.55-0.66 h; (c) Polar and lateral view, four cell stage, oil droplets two thirds of the way to the vegetal pole, 2 h; (d) Blastodisc with blastocoele, 3-4 h; (e) Blastoderm flattening and beginning to spread downward over yolk, 5 h; (f) The germ ring, embryonic shield and axis are visible, 14 h; (g) Blastoderm covering yolk except for yolk plug, neural groove visible, 20.5-21.5 h; (h) Early embryo formed, optic vesicles apparent on head 23.5-24.5 h; (i) Optic vesicles have enlarged and Kupffer's vesicle is seen clearly, 26 h; (j) Caudal somites and pericardial cavity apparent, 27-27.5 h; (k) Heart appears in pericardial cavity. Flow of plasma is indicated by the arrows, 47-47.5 h; (l) Eye pigmented and lens formed, otic vesicles are visible, melanophores on head, tail and yolk sac. (Diagram shows melanophore pattern of *M. nigrans*), 54.5-55 h; (m) Mouth present edged with melanophores, yellow vesicle marks position of the liver, branchial arches apparent. (Diagram shows melanophore pattern of *M. s. inornata,* expanded by strong light), 98-100 h. B, blastodisc; Bc, blastocoele; Bl, blastoderm; Br, branchial arches; C1, one cell; C4, four cells; Ch, chorion; E, eye; Ea, embryonic axis; Es, embryonic shield; F, filaments; Gr, germ ring; H, heart; Kv, Kupffer's vesicle; L, lens; Li, liver; M, mouth; Mel, melanophores; Ng, neural groove; O, oil droplets; Oc, optic cup; Ov, optic vesicle; Otv, otic vesicle; Pc, pericardial cavity; Pf, pectoral fin; Ps, perivitelline space; S, somites; T, tail; Tb, tail bud; Y, yolk; Yp, yolk plug; Ys, yolk sac.

STAGE	TIME	
	M. nigrans	*M. s. inornata*
10. Optic vesicles apparent. Head of developing embryo is close to the oil droplets. (Fig. 1h.)	23.5 h.	24.5 h.
11. The optic vesicles are prominent features on the head. Kupffer's vesicle (Armstrong and Child, 1965; Ruck, 1980) is clearly visible. (Fig. 1i).	26 h.	26 h.
12. First caudal somites visible. The pericardial cavity is obvious below the head. (Fig. 1j).	27.5 h.	27 h.
13. Brain ventricles have enlarged. Optic cup is apparent, but no eye lens is visible.	30.5 h.	32 h.
14. First melanophores appear on the yolk sac.	38 h.	38.5 h.
15. Melanophores start to darken the optic cup and they also appear along the sides of the body and head.	47 h.	46.5 h.
16. Plasma, but no red blood cells, circulates through the heart and yolk sac vessels. The heart is a straight tube and the pulse rate can be measured from the movement of the pericardial cavity. The pulse is slow at first, but increases as the embryo develops. (Fig. 1k).	47.5 h.	47 h.
17. Flexing of the body and tail occurs. The tail extends free from the yolk sac. Embryo is two-thirds around yolk sac.	51.5 h.	49.5 h.
18. Otic vesicles visible. Red blood cells circulate through the heart and yolk sac vessels. Discontinuity of the blood flow through the heart indicates the boundary of the developing chambers. Fig. 1l)	55 h.	54.5 h.
19. Pectoral fin buds appear as small semicircular ridges. The eye lens is fully formed and retinal pigment is fairly dense. The swim bladder appears as a small vesicle just below junction of body and tail.	72 h.	72 h.

STAGE	TIME	
	M. nigrans	*M. s. inornata*
20. Branchial arches are first seen. The yolk sac is reduced. The tail which moves frequently always comes to rest in the equatorial plane past the anterior part of the head.	96 h.	96.5 h.
21. The upper jaw is apparent, later edged by a row of melanophores. A yellow vesicle below and behind the left pectoral fin marks the position of the liver. The pectoral fins have grown and move freely. (Fig. 1m).	98 h.	100 h.
22. Meckel's cartilage can be seen as the lower jaw forms. Yellow colour spreads throughout the dorsal surface of the head.	102 h.	103 h.
23. The mouth opens and shuts frequently. Both upper and lower jaws are edged with melanophores. The heart chambers are well defined. The yellow colour has spread along the body and tail. The yolk sac is not visible when the embryo is viewed from the dorsal surface. Turning of the whole embryo within the chorion is frequent, particularly when the egg is subjected to strong light. Oil droplets are still present in the anterior of the yolk sac but are fewer in number.	128 h.	130 h.
24. Hatching. Prior to hatching the embryos lie with the tail coiled to one side rather than around the equatorial plane of the yolk sac. In *M. s. inornata* a spherical protuberance appears on the chorion directly in front of the mouth a few seconds before hatching.	155-159 h.	151-152 h.

The timing of the development of the embryos of the two species is almost parallel when conditions are constant, but the hatching of *M. s. inornata* occurs several hours earlier than in *M. nigrans*. The embryos are distinguishable by their melanophore pattern (Fig. 1l and 1m), although there is slight variability in this pattern within each species. In both species, the size of the melanophores increases under strong light to produce continuous dark patches.

CHANGE IN RATE OF HEART BEAT DURING EMBRYONIC DEVELOPMENT

Rhythmic contractions of the pericardial cavity indicate the onset of circulation of plasma through the heart and yolk sac vessels. Red blood cells appear in the plasma seven to eight hours after the onset of circulation. The colour of the plasma becomes faintly pink, but gradually darkens to red as more red blood cells develop.

The pulsations of the pericardial cavity are initially slow, but increase in rate as the embryos develop. The correlation between increasing heart rate and age of embryo is $r = 0.852$ ($p < 0.001$) ($N = 39$) for *M. s. inornata* and for *M. nigrans,* $r = 0.841$ ($p < 0.001$) ($N = 38$).

DISCUSSION

In contrast to previous reports (Breder and Rosen, 1966; Lake, 1971; Beumer, 1979) it appears that melanotaeniids, specifically *M. nigrans* and *M. s. inornata* shed 50 to 100 eggs at each spawning. Survival of eggs is reduced by predation activity of the parents.

K. A. Bishop (pers. comm.) reports that melanotaeniids in the Alligator Rivers region may spawn only when conditions are favourable. Beumer (1979) suggests that *M. s. splendida* may spawn throughout the year. Following photo-period experiments, Beumer also indicated that day length could have been a factor which induced spawning. In this study both *M. nigrans* and *M. s. inornata* spawned every day during the summer months with a decline in spawning activity at the onset of autumn as day length shortened. Since the temperature was maintained at 26° ± 1°C, with food supply readily available, the photo-period could be regarded as a contributing factor to the decline in spawning activity. However, a number of fish maintained under a 12-hour light/dark cycle also stopped spawning, suggesting either an endogenous rhythm or that the female became totally spent after spawning daily for several months.

A wide pH range does not appear to affect reproduction of melanotaeniids. The water in which the fish were kept in our laboratory (pH 7.8) was more alkaline than that of their natural habitat (pH 3.9-6.7; K. A. Bishop, pers. comm.), yet the fish continued to thrive and spawn, and embryo development was normal.

Since *M. nigrans* and *M. s. inornata* can easily be kept and bred, they could be useful in laboratory experiments in which the relationships between reproduction and environmental requirements (such as photo-periodicity, temperature and pH) are studied.

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